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(54) **Liposomes on which adsorption of proteins is inhibited.**

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Description

Background of the Invention

Technical Field

The present invention relates to liposomes on which adsorption of proteins is inhibited and which are agglutination-free and to methods for preparing the same.

Prior Art

Use of liposomes as a carrier for water-soluble or fat-soluble drugs has widely been attempted (Gregoriadis, et al., Ann. N.Y. Acad. Sci., 446, 319 (1985)). Use of liposomes as artificial erythrocytes by incorporating hemoglobin, the oxygen carrier for animals, in the inner aqueous space of liposomes has also been attempted (Japanese Patent Application Laid-Open to Public 178521/1987). Liposome membrane-constituting materials of the liposomes used in these attempts, however, were those composed only of natural or synthetic lipids such as phospholipids and cholesterol.

In order to use liposomes as a carrier for drugs it is necessary to introduce the liposomes into blood vessels in the living body. However, the liposomes composed only of lipids which were conventionally employed were encountered with problems of adsorbing plasma-constituting proteins of the living body (for example, albumin, globulin and fibrinogen) which results in mutual agglutination of the liposomes. The problems were considerable especially of the liposomes which particle size exceeds 0.1 μm. Particle size of the liposomes generally employed is usually 0.1 μm - 1 μm. The particle size as it is will be of no obstacle in passing through the blood vessels in the living body because the capillary blood vessels have inner diameter as large as several μm. However, if the liposomes are agglutinated by adsorbing plasma-constituting proteins, size of the agglutinates becomes tens of micrometers. If the agglutination occurs in the blood vessel, agglutinates of the liposomes will plug the blood vessel to inhibit blood flow possibly causing death of the living body.

Particularly when liposomes are used as artificial erythrocytes, a large dose of liposomes should be administered so that the problem of liposome agglutination in plasma was not negligible. Heretofore, however, there has been developed no technique at all for preventing the agglutination of liposomes in plasma.

In addition, when liposomes are introduced into the living body, antibody protein (immunoglobulin) to the liposome which is an antigen will be adsorbed on the liposomes to produce foreign body recognition in the phagocytes (macrophage) with a result that the liposomes will be included in the macrophage and disappear within a short period of time. Therefore, inhibition of the protein adsorption on liposomes could also delay disappearance of the liposomes in plasma.

It is also noted that hemoglobin concentration in natural erythrocytes is approximately 30%; as volume ratio of erythrocytes to the whole blood (hematocrit) is approximately 50%, hemoglobin concentration in the whole blood is approximately 15%. Accordingly, in the case of artificial erythrocytes which are formed by enclosing hemoglobin in the liposome smaller in particle size than natural erythrocytes, volume ratio of artificial erythrocytes in an artificial erythrocyte suspension will exceed 50% when hemoglobin concentration in the artificial erythrocyte suspension is 15%, unless an aqueous solution of hemoglobin with a hemoglobin concentration of 30% or more is subjected to liposome formation. Such suspension, which is poorly fluidized, will produce adverse effects upon circulatory dynamism when administered. In this respect, it is desirable to encapsulate a large amount of hemoglobin in the inner aqueous space of liposomes using lipid in an amount as small as possible. In other words, a method for preparing artificial erythrocytes with a high encapsulation efficiency is desirable. By the dialysis method or the reverse phase method, however, it is difficult to form liposomes of an aqueous solution of hemoglobin with a higher hemoglobin concentration (30% or more) and a higher viscosity. Also by the lamina method in which a liposome-forming lipid is uniformly dissolved in an organic solvent, then the organic solvent is removed and an aqueous solution is added to the lamina of the lipid thus formed to a dispersion, the hydration and dispersion cannot easily be accomplished by the addition of an aqueous solution because the liposome-forming lipid after removal of the organic solvent has been solidified or nearly in loss of fluidity. When the aqueous solution is an aqueous solution of hemoglobin with a high concentration, proportion of the water combined with the globin protein is high, and amount of the free water available for hydration of the lipid is small. Thus, liposome formation at a high efficiency was difficult. Therefore, an object of the invention is to provide, liposomes on which adsorption of proteins in plasma is inhibited and methods for preparing the same. A further object of the invention is to provide a method for preparing artificial erythrocytes comprising forming liposomes of a highly-concentrated hemoglobin at a high efficiency.

GB-A-2 185 397 discloses particles of drugs coated with a material to form a composite particle which substantially prevents the take-up of the composite particle by the liver. The structure of

said coating material includes hydrophilic and hydrophobic domains.

European patent applications EP-A-0 118 316, EP-A-0 072 111 and EP-A-0 220 797 disclose the preparation of liposomes using PEG-bond phospholipids. PEG in the thus prepared liposomes extends both outwardly and inwardly from their membrane, thus limiting the inner space to entrap drugs.

Summary of the Invention

As a result of extensive studies in order to achieve the above-mentioned objects we have found that adsorption of proteins in plasma on the surface of liposomes can be prevented by incorporating a specific agent for inhibiting adsorption of proteins into lipid layer of the liposome, eventually preventing agglutination of the liposomes each other and further facilitating hydration of the lipid even when artificial erythrocytes are prepared with an aqueous solution of hemoglobin at a high concentration thereby enabling formation of liposomes of a highly-concentrated hemoglobin at a high efficiency. The present invention was completed on the basis of the above findings.

According to the invention, there are provided liposomes on which adsorption of proteins is inhibited and methods for preparing the same as described below.

1) A liposome on which adsorption of proteins is inhibited which comprises a PEG-bound phospholipid wherein the PEG moiety is bonded to a phospholipid present in the liposome membrane and extends only outwardly from the surface of said liposome.

2) A liposome according to item 1 wherein hemoglobin is enclosed within the liposome.

3) The liposome according to item 1, wherein the phospholipid is phosphatidylethanolamine.

4) A process for the preparation of a liposome which comprises adding a PEG-bound phospholipid to a liposome suspension.

5) A process for the preparation of a liposome which comprises forming a liposome containing phospholipid having a reactive functional group as a liposome-membrane constituting lipid, mixing the resulting liposome with PEG activated so as to bind with said phospholipid so that one end of the PEG is bonded to said phospholipid and the other end extends outwardly.

6) The process according to item 5, wherein the phospholipid is phosphatidylethanolamine.

7) A method for preventing mutual agglutination of liposomes which comprises fixing PEG-bound phospholipid on a liposome surface.

Detailed Description of the Invention

The agents for inhibiting adsorption of proteins on the liposome surface or the agents for preventing agglutination of liposomes in the present invention are compounds which have a hydrophobic moiety at one end and a hydrophilic macromolecular chain moiety at the other end.

As preferred examples of the hydrophobic moiety are mentioned alcoholic radicals of a long chain aliphatic alcohol, a sterol, a polyoxypropylene alkyl or a glycerin fatty acid ester and phospholipids. As preferred examples of the hydrophilic macromolecular chain moiety are mentioned polyethylene glycols.

Used in the invention are non-ionic surface-active agents of PEG addition type in which a polyethylene glycol (called PEG hereinbelow) and an alcoholic radical of the hydrophobic moiety are bound by ether bond or PEG-bound phospholipids in which PEG and a phospholipid are covalently bound.

The polyethylene glycol-bound phospholipid in the invention is a molecule of such a structure that polyethylene glycol (PEG) is covalently bound with the hydrophilic moiety (polar head) of a phospholipid which contains one or more PEG chains per molecule. The end of the PEG chain that has not been bound with the phospholipid may also be hydroxyl group or an ether with a short chain such as with methyl or ethyl or an ester with a short chain such as with acetic acid or lactic acid.

In order to achieve the objects of the invention, PEG chain length in the PEG-bound phospholipid molecule is desirably in the range of 5 - 1000 moles, more preferably 40 - 200 moles in terms of the average degree of polymerization. Below the above-defined range, the effect of preventing agglutination of liposomes in plasma will hardly be produced. Beyond the above-defined range, water-solubility of the PEG-bound phospholipid will be too high to be readily fixed inside the liposome membrane.

In order to produce a covalent bond between PEG and a phospholipid a reaction-active functional group is necessary at the polar moiety of the phospholipid. The functional group includes amino group of phosphatidylethanolamine, hydroxyl group of phosphatidylglycerol, carboxyl group of phosphatidylserine; the amino group of phosphatidylethanolamine is preferably used.

For the formation of a covalent bond between the reaction-active functional group of a phospholipid and PEG are mentioned a method employing cyanuric chloride, a method employing a carbodiimide, a method employing an acid anhydride, a method employing glutaraldehyde. The method employing cyanuric chloride (2,4,6-trich-

loro-s-triazine) is preferably used for binding the amino group of phosphatidylethanolamine with PEG. For example, treatment of monomethoxypolyethylene glycol and cyanuric chloride by known reaction procedures affords 2-O-methoxypolyethylene glycol-4,6-dichloro-s-triazine (activated PEG1) or 2,4-bis-(O-methoxypolyethylene glycol)-6-chloro-s-triazine (activated PEG2) [Y. Inada, et al., Chem. Lett., 7, 773-776 (1980)]. Binding of these with the amino group by a dehydrochloric acid condensation reaction yields a phospholipid with PEG covalently bound with the polar head of phosphatidylethanolamine. In the above reaction there is contained one PEG chain in one phospholipid molecule when employing activated PEG1 and two PEG chains with activated PEG2. Phospholipids bound with PEG via amide bond is also produced by reacting monomethoxy PEG with succinic anhydride to introduce a carboxyl group into the end of the PEG and reacting the product with phosphatidylethanolamine in the presence of a carbodiimide.

In order to prepare a liposome with the PEG-bound phospholipid, wherein the PEG moiety extends inwardly and outwardly from the surface of said liposome (not part of the present invention) a PEG-bound phospholipid may uniformly be mixed with a liposome-forming lipid in advance, and the lipid mixture may be treated by a conventional method to form liposomes. The liposome-forming lipids as herein referred to contain as the main component phospholipids obtained from natural materials such as egg yolk and soybean or those which are produced by organic chemical synthesis used alone or in combination. Representative are phosphatidylcholine, sphingomyelin, phosphatidylethanolamine and phosphatidylserine. In addition, sterols such as cholesterol and cholestanol as a membrane-stabilizing agent, phosphatidic acid, dicetyl phosphate and higher fatty acids as a charged substance and other additives may be added. Mixing ratio of the PEG-bound phospholipid with the liposome-forming lipid is 0.1 - 50 mol%, preferably 0.5 - 20 mol% and more preferably 1 - 5 mol% in terms of the molar ratio to the phospholipid of the main component. Below the above-defined range, the effect of preventing agglutination of liposomes in plasma will not be sufficiently high. Beyond the above-defined range, solubilizing capacity of the PEG-bound phospholipid will cause instabilization of the liposome.

In effecting in advance uniform mixing of the liposome-forming lipid with the PEG-bound phospholipid, for example, the two may be dissolved in a volatile organic solvent and then the organic solvent removed by evaporation. If a fat-soluble drug is to be contained in the liposomes, it may be mixed with the liposome-forming lipid during the above procedures. Formation of liposomes from the mixed lipids thus obtained may be carried out according to a liposome formation method usually employed. For example, any of such methods as shaking, sonication and French pressure cell may be employed. Liposomes of particle sizes between 0.1 μm and 1 μm are produced allowing for carrying a sufficient amount of a water-soluble drug or physiologically active substance in the inner aqueous space, provided that the above-mentioned PEG-bound phospholipid is used within the above-defined ranges. The PEG-bound phospholipid is contained in the lipid layer of liposomes thus obtained, but the content is not necessarily the same as that based upon the proportion originally mixed with the lipid. If water solubility of the PEG-bound phospholipid is high, part of it will possibly be eluted into the aqueous phase outside the membrane. Although the form of the PEG-bound phospholipid present in the lipid membrane of liposome is not clear, it is believed that the hydrophobic moiety of the PEG-bound phospholipid is present in the hydrophobic region of the liposome membrane, and the hydrophilic PEG chain is present from the hydrophilic region in the membrane over to the aqueous medium outside the membrane. It follows therefore that the PEG chain of the PEG-bound phospholipid in the liposome obtained by this method is present in both of the outer aqueous phase and the inner aqueous space of the liposome.

The PEG-bound phospholipid of the invention need not necessarily give a clear solution when dissolved in water. However, if the PEG-bound phospholipid of the invention is uniformly dissolved in water, the liposome of the invention may also be prepared by an alternative method. As a matter of fact, liposomes containing the PEG-bound phospholipid in the lipid layer may also be prepared as follows: To a suspension of liposomes carrying a water-soluble or fat-soluble drug (which have been prepared by a conventionally employed liposome formation method) is added the PEG-bound phospholipid of the invention either as it is or in aqueous solution. In this case, the PEG-bound phospholipid appears to be in dispersion in the form of micelle-like molecular aggregates in the aqueous solution. When liposomes are co-existent in the dispersion, the hydrophobic moiety in the PEG-bound phospholipid molecule is fixed in the hydrophobic region in the liposome membrane by hydrophobic interaction thereby taking a structure in which the hydrophilic PEG chain is exposed on the surface of liposomes on the side of the outer aqueous phase only.

Addition of the PEG-bound phospholipid in aqueous solution may be made at the critical

micelle concentration or higher. At a lower concentration, however, amount of the phospholipid adsorbed on the liposome will not be sufficient to maintain the effect of preventing agglutination of liposomes in plasma. At a too high concentration, the liposome will be so unstable as eventually to cause leakage of the water-soluble drug carried in the inner aqueous space. Therefore, the concentration is preferably 0.01 - 20%, more preferably 0.05 - 20% in terms of the concentration in the liposome suspension.

Liposomes containing the PEG-bound phospholipid in the lipid layer can also be prepared by an alternative method. As a matter of fact, liposomes containing a phospholipid with a reaction-active functional group are prepared by a conventional method, and subsequently a PEG activated at one end is added to the outer solution of the liposomes to allow for binding with the phospholipid. For example, liposomes containing 1 - 50 mol% of phosphatidylethanolamine in the whole phospholipid are prepared, activated PEG2 in a basic buffer solution (pH 9 or higher) is added at a concentration of 1 - 20% and the mixture is allowed to react at room temperature for 1 - 24 hours. There is formed a structure in which the hydrophilic PEG chain is exposed on the surface on the side of the outer aqueous phase of the liposomes.

When artificial erythrocytes are prepared, mixing ratio of the non-ionic surface active agent to the liposome-forming lipid is preferably 0.5 - 30% by weight. Below the above-defined range, formation of hemoglobin liposomes will hardly be achieved at a high efficiency. Beyond the above-defined range, solubilizing capacity of the non-ionic surface active agent will unstabilize the artificial erythrocytes formed.

The liposome-forming lipid used in the invention is phospholipids obtained from natural materials such as egg yolk and soybean or those which are produced by organic chemical synthesis. They are used as the main component either alone or in combination. Representative are phosphatidylcholine (lecithin), sphingomyelin, phosphatidylethanolamine and phosphatidylserine. In addition, sterols such as cholesterol and cholestanol as a membrane-stabilizing agent, phosphatidic acid, dicetyl phosphate and higher fatty acids as a charge-providing substance and other additives may also be added.

If the phospholipid contains an unsaturated bond, there occur such special problems that lipid peroxides generated by peroxidation reaction of the unsaturated bond may be toxic, and the enclosed hemoglobin is liable to oxidative degradation. Therefore, hydrogenation products to the unsaturated group are preferably used. For example, hydrogenated egg yolk lecithin, hydrogenated soybean lecithin are mentioned as hydrogenated natural phospholipid readily available. When such a hydrogenated natural phospholipid is employed as the main component, the phase transition temperature is as high as about 50°C. In general, liposomes are hardly formed unless the operation is carried out at the phase transition temperature or higher. However, hemoglobin will be heat degraded if formation of hemoglobin liposomes is operated at 40°C or higher. If sterols are contained in the liposome-forming lipid, there is no definite phase transition temperature for the whole lipid mixture, and artificial erythrocytes can be prepared satisfactorily even when operated at a temperature below the phase transition temperature of the lipid main component. Higher fatty acids are preferably employed as a charge-providing substance which is usually contained in order to prevent mutual agglutination of the formed artificial erythrocytes. Adequately, mixing ratios in these liposome-forming lipids are 0.2 - 1 part by weight of sterols and 0.05 - 0.2 parts by weight of higher fatty acids per part by weight of the phospholipid.

In order to prepare a mixture of a non-ionic surface active agent and a liposome-forming lipid the two may uniformly be dissolved in a volatile organic solvent capable of uniformly dissolving the non-ionic surface active agent and the liposome-forming lipid and then the organic solvent removed by such a method as evaporation, freeze-drying or spray-drying.

In order to form artificial erythrocytes from the mixed lipid obtained, said mixed lipid may be hydrated and dispersed in an aqueous solution of hemoglobin. Whereas the hydration and dispersion may be effected merely by mechanically mixing the two, it is desirable to add high pressure-delivery treatment using such a machine as a French pressure cell. Hemoglobin concentration in the aqueous solution of hemoglobin is preferably 30 - 60%. Below the above-defined range, encapsulation efficiency of the hemoglobin will be low. Beyond the above-defined range, viscosity of the aqueous solution of hemoglobin will be so much increased that the hydration and dispersion will be difficult even when a non-ionic surface active agent is added.

In the method for preparing artificial erythrocytes according to the invention in which a liposome-forming lipid with hydrogenated phospholipids, sterols or higher fatty acids mixed and an aqueous solution of hemoglobin in the above-defined range are used, there are produced almost none of the artificial erythrocytes with particle sizes of 0.01 - 0.03 μm having very low hemoglobin encapsulation efficiency, but for the most part, artificial erythrocytes with particle sizes

of 0.1 μm or larger having high hemoglobin encapsulation efficiency.

In the lipid layer of the artificial erythrocytes thus obtained is contained the non-ionic surface active agent content of which is not necessarily be the same as that based upon the initial mixing ratio with the lipid. In case where water solubility of the non-ionic surface active agent is high, part of it will possibly be eluted into the aqueous phase outside the membrane.

The invention will be described in more detail below with reference to Examples.

Example 1

(The liposomes described in this example are not part of the present invention.)

In 50 ml of dehydrated chloroform were dissolved 150 mg of dipalmitoylphosphatidylethanolamine and 2.5 g of activated PEG2 (average molecular weight of PEG 5,000 x 2, manufactured by Seikagaku Kogyo K.K.). To the solution was added 2 g of sodium carbonate, and the mixture was allowed to react overnight at room temperature. After confirming completion of the reaction by disappearance of the ninhydrin color reaction the reaction mixture was filtered, and hexane was added to the filtrate for purification by reprecipitation. The purified product was dried in vacuo to obtain a PEG-bound phospholipid.

In 20 ml of dichloromethane were dissolved 630 mg of hydrogenated egg yolk lecithin, 317 mg of cholesterol, 53 mg of myristic acid and 150 mg of the above-obtained PEG-bound phospholipid. The organic solvent was removed by evaporation. To the mixed lipid thus obtained was added 20 ml of 50% aqueous solution of hemoglobin. The mixture was blended by shaking followed by French pressure cell under a pressure of 250 kg/cm^2. The treatment was repeated ten times, and the liquor obtained was 1:10 diluted with physiological saline solution and subjected to centrifugal separation (17,000 r.p.m. for 30 min.).

The liposome precipitates were subjected to additional centrifugal washing with two portions of 140 ml of physiological saline solution. The liposome precipitates after the washing were suspended in physiological saline solution to a hemoglobin concentration of 5%. Average particle size of the liposomes thus obtained was 0.2 μm. With 0.1 ml of the liposome suspension was mixed 0.5 ml of citrate-containing human plasma. The mixture was observed under optical microscope to find almost none of liposome agglutinates exceeding 1 μm in size.

Example 2

In 20 ml of dichloromethane were dissolved 630 mg of hydrogenated egg yolk lecithin, 317 mg of cholesterol and 53 mg of myristic acid. The organic solvent was removed by evaporation. To the mixed lipid thus obtained was added 20 ml of 50% aqueous solution of hemoglobin. The mixture was blended by shaking followed by French pressure cell under a pressure of 500 kg/cm^2. The treatment was repeated ten times, and the liquor obtained was 1:10 diluted with physiological saline solution and subjected to centrifugal separation (17,000 r.p.m. for 30 min.). The liposome precipitates were subjected to additional centrifugal washing with two portions of 140 ml of physiological saline solution. The liposome precipitates after the washing were suspended in physiological saline solution to a hemoglobin concentration of 5%. Average particle size of the liposomes thus obtained was 0.2 μm. With 0.1 ml of the liposome suspension was mixed 0.5 ml of citrate-containing human plasma. The mixture was observed under optical microscope (x 400) to find liposomes completely agglutinated. Size of the agglutinates exceeded 50 μm.

To 1 ml of the above-prepared liposome suspension adjusted to a hemoglobin concentration of 5% was added 9 ml of physiological saline solution containing 1% of the PEG-combined phospholipid obtained in Example 1. The mixture was allowed to stand at room temperature for 30 min., then 1:10 diluted with physiological saline solution and subjected to centrifugal separation (17,000 r.p.m. for 30 min.). The liposome precipitates were subjected to additional centrifugal washing with two portions of 140 ml of physiological saline solution. The liposome precipitates after the washing were suspended in physiological saline solution to a hemoglobin concentration of 5%. With 0.1 ml of the liposome suspension was mixed 0.5 ml of citrate-containing human plasma. The mixture was observed under optical microscope (x 400) to find almost none of the liposome agglutinates exceeding 1 μm in size.

Example 3

The same procedures as in Example 2 were repeated except that hydrogenated soybean lecithin containing 30 mol% of phosphatidylethanolamine was used in place of the hydrogenated egg yolk lecithin to obtain hemoglobin-containing liposomes. To 1 ml of a suspension of the above-prepared liposomes adjusted with 0.1 M borate buffer solution (pH 10) to a hemoglobin concentration of 5% was added 100 mg of activated PEG2. The mixture was allowed to react

overnight at room temperature. The reaction mixture was 1:10 diluted with physiological saline solution and subjected to centrifugal separation (17,000 r.p.m. for 30 min.). The liposome precipitates were subjected to additional centrifugal washing with two portions of 140 ml of physiological saline solution. The liposome precipitates after the washing were suspended in physiological saline solution to a hemoglobin concentration of 5%. With 0.1 ml of the liposome suspension was mixed 0.5 ml of citrate-containing human plasma. The mixture was observed under optical microscope (x 400) to find almost none of the liposome agglutinates exceeding 1 μm in size.

Example 4

To a solution of 50 g of monomethoxy PEG5000 (manufactured by Union Carbide) in 250 ml of 1,2-dichloromethane were added 5 g of succinic anhydride and 4 ml of pyridine. The mixture was boiled under reflux for 4 days. The reaction mixture was filtered, subjected to evaporation and dissolved in 100 ml of distilled water. The aqueous phase was washed with ether and then extracted with 100 ml of chloroform. After evaporated the residue was recrystallized from ethyl acetate to give monocarboxy-terminated PEG. In 30 ml of chloroform were dissolved 725 mg of the PEG, 100 mg of dipalmitoylphosphatidylethanolamine and 30 mg of dicyclohexylcarbodiimide. The solution was allowed to react overnight at 50°C. The reaction mixture was subjected to re-precipitation with 300 ml of hexane. There was obtained phospholipid bound via amide bond with PEG. The same results as in Examples 1 and 2 were produced in an experiment using the phospholipid.

Claims

1. A liposome on which adsorption of proteins is inhibited which comprises a PEG-bound phospholipid wherein the PEG moiety is bonded to a phospholipid present in the liposome membrane and extends only outwardly from the surface of said liposome.

2. The liposome according to claim 1 wherein hemoglobin is enclosed within the liposome.

3. The liposome according to claim 1 or 2, wherein the phospholipid is phosphatidylethanolamine.

4. A process for the preparation of a liposome according to anyone of claims 1 to 3 which comprises adding a PEG-bound phospholipid to a liposome suspension.

5. A process for the preparation of a liposome according to anyone of claims 1 to 3 which comprises forming a liposome containing phospholipid having a reactive functional group as a liposome-membrane constituting lipid, mixing the resulting liposome with PEG activated so as to bind with said phospholipid so that one end of the PEG is bonded to said phospholipid and the other end extends outwardly.

6. The process according to claim 5, wherein the phospholipid is phosphatidylethanolamine.

7. A method for preventing mutual agglutination of liposomes which comprises fixing PEG-bound phospholipid on a liposome surface.

Patentansprüche

1. Liposom, auf dem eine Adsorption von Proteinen gehemmt ist, umfassend ein PEG-gebundenes Phospholipid, bei dem die PEG-Einheit an ein in der Liposommembran vorhandenes Phospholipid gebunden ist und sich lediglich von der Liposomoberfläche nach außen erstreckt.

2. Liposom nach Anspruch 1, wobei in dem Liposom Hämoglobin eingeschlossen ist.

3. Liposom nach Anspruch 1 oder 2, wobei das Phospholipid aus Phosphatidylethanolamin besteht.

4. Verfahren zur Herstellung eines Liposoms nach einem der Ansprüche 1 bis 3 durch Zugabe eines PEG-gebundenen Phospholipids zu einer Liposomsuspension.

5. Verfahren zur Herstellung eines Liposoms nach einem der Ansprüche 1 bis 3 durch Ausbilden eines Liposoms mit einem Phospholipid mit reaktionsfähiger funktioneller Gruppe als Liposommembran bildendes Lipid, Vermischen des erhaltenen Liposoms mit PEG, das derart aktiviert ist, daß es eine Bindung mit dem Phospholipid dergestalt eingeht, daß ein Ende des PEG an das Phospholipid gebunden ist und sich das andere Ende nach außen erstreckt.

6. Verfahren nach Anspruch 5, wobei das Phospholipid aus Phosphatidylethanolamin besteht.

7. Verfahren zur Verhinderung einer wechselseitigen Agglutination von Liposomen durch Fixie-

ren eines PEG-gebundenen Phospholipids auf einer Liposomoberfläche.

Revendications

1. Liposome sur lequel l'adsorption de protéines est inhibée, qui comprend un phospholipide auquel est lié du PEG, le motif PEG étant lié à un phospholipide présent dans la membrane du liposome et s'étendant uniquement à l'extérieur à partir de la surface dudit liposome.

2. Liposome selon la revendication 1, dans lequel de l'hémoglobine est incluse à l'intérieur du liposome.

3. Liposome selon la revendication 1 ou 2, dans lequel le phospholipide est de la phosphatidyléthanolamine.

4. Procédé de préparation d'un liposome selon l'une quelconque des revendications 1 à 3, lequel comprend l'addition d'un phospholipide auquel est lié du PEG à une suspension de liposomes.

5. Procédé de préparation d'un liposome selon l'une quelconque des revendications 1 à 3, lequel comprend la formation d'un liposome oontenant un phospholipide ayant un groupement fonctionnel réactif en tant que lipide constituant la membrane du liposome, le mélange du liposome obtenu avec du PEG activé de façon à le lier audit phospholipide de telle sorte qu'une extrémité du PEG soit liée audit phospholipide et que l'autre extrémité s'étende vers l'extérieur.

6. Procédé selon la revendication 5, dans lequel le phospholipide est de la phosphatidyléthanolamine.

7. Procédé de prévention de l'agglutination mutuelle de liposomes, lequel comprend la fixation d'un phospholipide auquel est lié du PEG à la surface d'un liposome.